Effects of Dietary Inulin Supplementation Levels on Cecal and Rectal Microbiota Structure and Abundance of Major Bacterial Groups in Broiler Chickens: Postprint

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Date: 2017-10-10T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary inulin supplementation levels on the cecal and rectal microbial community structure and the quantities of major bacterial groups in broiler chickens. A single-factor completely randomized design was adopted, using 300 1-day-old Arbor Acres broiler chickens that were randomly divided into 5 groups with 6 replicates per group and 10 birds per replicate. The five groups of broiler chickens were fed experimental diets supplemented with 0 (control group), 0.5, 1.5, 2.5, and 5.0 g/kg inulin in the basal diet, respectively. The experimental period lasted for 6 weeks. PCR-denaturing gradient gel electrophoresis (DGGE) and quantitative PCR (qPCR) techniques were used to determine the cecal and rectal microbial community structure and the quantities of major bacterial groups in 42-day-old broiler chickens. The results showed: 1) Dietary inulin supplementation levels had no significant effects on cecal and rectal microbial richness, cecal microbial Shannon index, or rectal microbial evenness in broiler chickens ($P>0.05$), but significantly decreased cecal microbial evenness and rectal microbial Shannon index ($P<0.05$). Sequence analysis of partial dominant bands on PCR-DGGE profiles indicated that dietary inulin supplementation promoted the proliferation of bacteria such as Eubacterium coprostanoligenes, Intestinimonas sp., and Enterococcus cecorum from the phylum Firmicutes in the cecum and rectum of broiler chickens. 2) Dietary inulin supplementation significantly affected the quantities of total bacteria and Escherichia coli in the cecum and rectum of broiler chickens ($P<0.05$). Quadratic curve analysis revealed that the quantities of total bacteria and Escherichia coli in the cecum were minimized at inulin supplementation levels of 2.71 and 2.66 g/kg, respectively; compared with the control group, dietary supplementation with 1.5, 2.5, and 5.0 g/kg inulin significantly decreased cecal Lactobacillus quantities ($P<0.05$), while dietary sup-

plementation with 0.5 g/kg inulin significantly increased rectal Lactobacillus quantities (P<0.05); when dietary inulin was supplemented at 5.0 g/kg, the quantities of Bifidobacterium in the cecum and rectum and Clostridium perfringens in the rectum were the highest, and differed significantly from the control group (P<0.05). In conclusion, under the conditions of this experiment, the inulin supplementation level of 2.5–5.0 g/kg was beneficial for improving intestinal microbial community structure and promoting the proliferation of beneficial bacteria in broiler chickens.

Full Text

Abstract

This experiment was conducted to investigate the effects of dietary inulin supplementation on the microbial community structure and major microbial populations in the cecum and rectum of broiler chickens. Using a single-factor completely randomized design, 300 one-day-old Arbor Acres broilers were randomly allocated into five groups with six replicates per group and ten broilers per replicate. The five groups were fed a basal diet supplemented with 0 (control), 0.5, 1.5, 2.5, and 5.0 g/kg inulin, respectively. The experimental period lasted for six weeks. PCR-denaturing gradient gel electrophoresis (DGGE) and quantitative PCR (qPCR) techniques were employed to determine the microbial community structure and major microbial populations in the cecum and rectum of 42-day-old broilers. The results showed that: 1) Dietary inulin supplementation had no significant effects on microbial richness in the cecum and rectum, cecal microbial Shannon-Wiener index, or rectal microbial evenness (P>0.05), but significantly decreased cecal microbial evenness and rectal microbial Shannon-Wiener index (P<0.05). Analysis of some dominant bands in the PCR-DGGE profiles indicated that dietary inulin promoted the proliferation of bacteria such as *Eubacterium coprostanoligenes*, *Intestinimonas sp.*, and *Enterococcus cecorum* from the phylum Firmicutes in both the cecum and rectum of broilers. 2) Dietary inulin supplementation significantly affected the populations of total bacteria and *Escherichia coli* in the cecum and rectum (P<0.05). Quadratic curve analysis revealed that the populations of total bacteria and *E. coli* in the cecum were minimized at inulin supplementation levels of 2.71 and 2.66 g/kg, respectively. Compared with the control group, dietary supplementation with 1.5, 2.5, and 5.0 g/kg inulin significantly decreased cecal *Lactobacillus* populations (P<0.05), while supplementation with 0.5 g/kg inulin significantly increased rectal *Lactobacillus* populations (P<0.05). When dietary inulin was supplemented at 5.0 g/kg, the populations of cecal and rectal *Bifidobacteria* and rectal *Clostridium perfringens* were highest and significantly different from the control group (P<0.05). In conclusion, under the conditions of this experiment, an inulin supplementation level of 2.5–5.0 g/kg was beneficial for improving intestinal microbial community structure and promoting the proliferation of beneficial bacteria in broilers.

Keywords: inulin; microbial community; microbial population; intestine; broilers

Introduction

The intestinal microbiota and its metabolism play crucial roles in regulating nutrition, health, and disease in broiler chickens, and nutritional intervention to modulate host gut microbial health has become a hotspot in animal nutrition research [**?**]. The beneficial functions of inulin in broiler intestines have been widely demonstrated, and its application is receiving increasing attention [**?**]. Inulin, also known as inulin, is a fructan composed of D-fructose molecules linked via β(1$→$2) glycosidic bonds, primarily derived from Jerusalem artichoke and chicory roots. Inulin is tasteless, amorphous, highly water-soluble, with a melting point of 178 °C, relative density of 1.35, and strong thermal stability. Sun et al. [**?**] reported that dietary supplementation with 3 g/kg inulin reduced cecal *E. coli* and *Salmonella* populations by 14.50% and 36.42%, respectively, and effectively decreased ammonia emission from broiler excreta. Studies have found that inulin from chicory roots significantly promoted the proliferation of beneficial bacteria such as *Bifidobacteria* and *Lactobacillus* in the ileum and cecum of broilers [**?**]. With increasing inulin supplementation, skatole concentration in the large intestine and feces of boars decreased, while *Clostridium perfringens* populations in the colon and rectum were reduced, suggesting that reduced skatole concentration might be associated with decreased *C. perfringens* populations and increased short-chain fatty acid content [**?**]. However, other studies have shown that dietary inulin supplementation had no significant effect on broiler intestinal microbiota [**?**]. Our previous research indicated that dietary inulin supplementation did not affect broiler performance, but supplementation at 5.0 g/kg significantly reduced indole and skatole concentrations in excreta, cecum, and rectum [**?**]. To further investigate its effects on broiler intestinal microbiota, this study was conducted to examine the effects of dietary inulin supplementation levels on cecal and rectal microbial community structure and major microbial populations.

1.1 Experimental Materials and Diets

The inulin used in this experiment was produced by a Shanghai company, with purity >92%, approximate molecular weight of 5,000, appearing as a white crystalline powder, and tasteless. The basal diet was a corn-soybean meal-based powder, and its composition and nutrient levels were the same as in reference [**?**].

1.2 Experimental Design and Management

A single-factor completely randomized design was adopted. Three hundred one-day-old Arbor Acres broilers were randomly divided into five groups with six replicates per group and ten broilers per replicate. Group I served as the control

group fed the basal diet, while groups II, III, IV, and V were fed the basal diet supplemented with 0.5, 1.5, 2.5, and 5.0 g/kg inulin, respectively. Broilers were housed in three-tier full-step cages with hot-air furnace heating, manual feeding, nipple drinkers, mechanical manure removal, and ad libitum access to feed and water. The experimental period lasted for six weeks. Vaccination and routine management were conducted according to standard practices.

1.3 Sample Collection

At 42 days of age, one broiler from each replicate (ensuring three males and three females per group) was selected, euthanized by jugular vein exsanguination, and immediately dissected. The cecum and rectum were separated, snap-frozen in liquid nitrogen, and stored at -80 °C for subsequent analysis.

1.4.1 Microbial Community Structure

1) DNA extraction. After thawing cecal and rectal samples in a 4 °C refrigerator, genomic DNA was extracted from 15 cecal and 15 rectal samples using the cetyltrimethylammonium bromide (CTAB) manual extraction method, with samples from two broilers (one female and one male) pooled for each extraction. 2) PCR amplification of bacterial 16S rDNA fragments: Using the sample genomic DNA as template, bacterial universal primers GC-338F (5 -CGCCCGGGGCGCGCCCCGGGGCGGGGCGGGGGCGCGGGGGGCCTACGGGAGGCAGCAG-3) and 518R (5 -ATTACCGCGGCTGCTGG-3) were used to amplify the V3 region of 16S rDNA. The PCR amplification system (50 L) consisted of: 10×PCR buffer 5 L, dNTP (2.5 mmol) 3.2 L, rTaq (5 U/ L) 0.4 L, GC-338F (20 mol) 1 L, 518R (20 mol) 1 L, template DNA 50 ng, and double-distilled water (ddH_2O) to 50 L. The PCR program was: 94 °C pre-denaturation for 5 min; 30 cycles of 94 °C denaturation for 1 min, 55 °C annealing for 45 s, and 72 °C extension for 1 min; final extension at 72 °C for 10 min. 3) Denaturing gradient gel electrophoresis (DGGE) analysis of PCR products. Ten microliters of PCR product were used for DGGE analysis using 7% polyacrylamide gel with a denaturing gradient of 35%–55% at 56 V and 60 °C in 1×TAE buffer for 16 h. After DGGE, silver staining was performed. PCR products were purified and recovered using the OMEGA DNA Gel Extraction Kit. The Quantity One software package was used for digital analysis of band numbers and densities in DGGE profiles. Cluster diagrams were constructed based on Dice' s coefficient (Cs), and principal component analysis (PCA) was performed using CANOCO 4.5 software. The calculation formulas were:

$$Cs = \frac{2j}{Nx + Ny}$$

$$H = -\sum_{i=1}^{S} p_i \ln(p_i)$$

$$E = \frac{H}{H_{\max}} = \frac{H}{\ln(S)}$$

Where: Cs, H, S, and E represent Dice' s coefficient, Shannon index, richness, and evenness, respectively; Nx is the number of bands in lane x; Ny is the number of bands in lane y; j is the number of common bands in two lanes; pi is the proportion of intensity of a single band to the total intensity of all bands in a sample; N is the density of all bands in a single lane; Ni is the density of the ith band; Hmax is the maximum value of H. 4) Recovery and sequencing of dominant bands in DGGE profiles. Dominant bands that were clear and well-separated in the DGGE profiles were completely excised with a sterile scalpel. Using 2 L of the recovered product as template, PCR amplification was performed with primers 338F/518R. The re-amplified DNA fragments were gel-recovered, purified, ligated into the pMD18-T vector, and transformed into DH5α competent cells. Positive clones were selected for sequencing, and the sequencing results were compared with sequences in GenBank to identify the bacterial types represented by the bands.

1.4.2 Major Microbial Populations

The primer sequences for total bacteria, *E. coli*, *Lactobacillus*, and *Bifidobacteria* were the same as in reference [**?**]. The primer sequences for *C. perfringens* were: forward primer 5 -GGGTTTCAACACCTCCGTG-3 , reverse primer 5 -CGATTAAGAGTAATGCAAGG-3 . Quantitative PCR (qPCR) was performed with 0.5 µL genomic DNA using a 20 µL system (10 µL 2×SG PCR MasterMix, 0.5 µL each of forward and reverse primers (10 µmol/L), 0.5 µL genomic DNA, and 8.5 µL ddH_2O) on a StepOnePlus™ Real-Time PCR System (USA). The reaction program was: pre-denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 20 s and 60 °C for 30 s. After confirming specific amplification by 1% agarose gel electrophoresis, two 50 µL PCR reactions were performed for product recovery. The amplified products were ligated to T-vector. Correctly sequenced plasmids were quantified, copy numbers were calculated based on molecular weight, and gradient dilutions were prepared as standards to generate standard curves. Sample DNA extracted in section 1.4.1 was diluted 10-fold and detected on the real-time PCR system following the same procedure as standard curve construction. Microbial populations in each sample were calculated based on Ct values and the standard curve, then converted to log values of bacterial copies per gram of sample.

1.5 Statistical Analysis

Data were processed and analyzed on a replicate basis using SPSS 17.0 software. One-way ANOVA was used for variance analysis, Duncan' s multiple range test was used for multiple comparisons, and linear and quadratic trends were analyzed using the Contrast procedure. $P<0.05$ was considered statistically significant. Experimental data were expressed as "mean ± standard deviation" .

2.1 Cecal Microbial Community Structure in Broilers

As shown in [Figure 1: see original paper], 50 bands were detected across 15 cecal samples. The numbers of bands in samples M1-1 to M5-3 were 20, 18, 20, 18, 19, 21, 19, 16, 11, 19, 15, 21, 18, 18, and 18, respectively. shows that dietary inulin supplementation had no significant effect on cecal microbial Shannon-Wiener index or richness ($P>0.05$), but the evenness in groups III and IV was significantly lower than in the control and group II ($P<0.05$). There was a significant quadratic relationship ($P<0.05$) between dietary inulin supplementation level (x) and cecal microbial evenness (y) in broilers, with the regression equation $y=0.243x^2-0.136x+0.974$ ($R^2=0.6717$). Cecal microbial evenness was minimized at an inulin supplementation level of 2.8 g/kg, with a value of 0.950.

2.2 Similarity of Cecal Microbial Community in Broilers

The PCA of PCR-DGGE profiles is shown in [Figure 2: see original paper]. Principal component 1 (PC1) and principal component 2 (PC2) explained 21.4% and 15.6% of the variance, respectively, accounting for 37.0% of the total DGGE profile information. PC1 separated samples into left and right sections, with samples from groups III and IV on the left, control and group V samples on the right, and group II samples distributed on both sides. PC2 separated samples into upper and lower sections, with group II samples in the upper section, group III samples in the lower section, control samples (except M1-2) in the upper section, group IV samples (except M4-1) in the lower section, and group V samples (except M5-3) in the lower section. Combined analysis of PC1 and PC2 revealed that sample M1-1 showed the greatest divergence from other samples, indicating the lowest similarity in gut microbiota.

The cluster analysis based on Cs is presented in [Figure 3: see original paper]. Among the 15 samples, sample M1-1 from the control group showed the lowest similarity to other samples, with a similarity coefficient of 0.44. Four clusters with high similarity were identified: M1-2 with M1-3, M2-2 with M2-3, M5-1 with M5-3, and M3-3 with M4-1, with similarity coefficients of 0.73, 0.76, 0.66, and 0.65, respectively. The clusters of M1-2 and M1-3 (from the control group) and M5-1 and M5-3 (from group V) showed relatively low similarity (similarity coefficient of 0.59), suggesting that inulin supplementation affected cecal microbial community structure in broilers. These results were consistent with the PCA findings.

2.3 Sequence Analysis of Dominant Bands in Cecal DGGE Profiles

Ten bands with common or specific characteristics in the cecal DGGE fingerprints were recovered, cloned, and sequenced. As shown in , all sequences showed 87%–100% similarity to 16S rDNA sequences in GenBank. Except for band 15, which had similar bacteria from the phylum Bacteroidetes, all others were from Firmicutes. Band 37 was common to samples M4-1, M4-3, and M5-2, and its most similar bacterium was *Eubacterium coprostanoligenes* from the genus *Eubacterium*, with 95% similarity. Bands 16 and 18 were common to all groups, with similar bacteria *Dielma fastidiosa* and *Faecalibacterium prausnitzii*, showing similarities of 87% and 96%, respectively.

2.4 Rectal Microbial Community Structure in Broilers

As shown in [Figure 4: see original paper], the numbers of bands in samples Z1-1 to Z5-3 were 24, 21, 19, 15, 19, 19, 11, 9, 14, 17, 13, 27, 18, 14, and 15, respectively. shows that dietary inulin supplementation had no significant effect on rectal microbial evenness or richness ($P>0.05$), but the Shannon-Wiener index in group III was significantly lower than in other groups except group V ($P<0.05$).

2.5 Similarity of Rectal Microbial Community in Broilers

The PCA of rectal microbial PCR-DGGE profiles is shown in [Figure 5: see original paper]. PC1 and PC2 explained 20.6% and 14.6% of the variance, respectively, accounting for 34.60% of the total DGGE profile information. PC1 separated samples into left and right sections, with control and group II samples on the right, groups III and V on the left, and group IV distributed on both sides. PC2 separated samples into upper and lower sections, with control samples in the upper section, groups II and V in the lower section, and groups III and IV distributed in both sections. Z3-3 and Z4-1 were closest in distance, while Z5-2 and Z1-2 (from group V and control group, respectively) were farthest apart. As shown in [Figure 6: see original paper], three clusters with high similarity were identified among the 15 samples: Z4-1 with Z3-3, Z1-1 with Z1-3, and Z3-1 with Z4-1 and Z3-3, with similarity coefficients of 0.72, 0.64, and 0.60, respectively. Z5-2 showed the lowest similarity coefficient (0.27) with other samples. These results indicated that group V had the lowest similarity in rectal microbial community compared with the other four groups.

2.6 Sequence Analysis of Dominant Bands in Rectal DGGE Profiles

Six bands with common or specific characteristics in the rectal DGGE fingerprints were recovered, cloned, and sequenced. As shown in , the sequences showed 95%–100% similarity to 16S rDNA sequences in GenBank. Band 2 was

common to samples Z3-1, Z3-3, and Z4-1, and its most similar bacterium was *Intestinimonas sp.*, with 95% similarity. Band 33 was common to samples Z2-3, Z3-1, Z4-3, and Z5-1, and its most similar bacterium was *Enterococcus cecorum*, with 100% similarity. Band 36 was common to samples Z2-2, Z2-3, and Z4-3, and its most similar bacterium was *Faecalibacterium prausnitzii*, with 96% similarity. Band 45 was common to samples Z2-3 and Z5-3, and its most similar bacterium was *Clostridium sordellii*, with 100% similarity.

2.7 Cecal Microbial Populations in Broilers

As shown in , the populations of total bacteria and *E. coli* in all treatment groups were significantly lower than in the control group ($P<0.05$). The cecal *Lactobacillus* populations in groups III, IV, and V were significantly lower than in the control and group II ($P<0.05$). The cecal *Bifidobacteria* populations in groups II and V were significantly higher than in the control and groups III and IV ($P<0.05$), with group V showing the highest population. Dietary inulin supplementation had no significant effect on cecal *C. perfringens* populations ($P>0.05$). Except for *Bifidobacteria*, all cecal bacterial populations showed significant linear relationships with dietary inulin supplementation level ($P<0.05$). Additionally, cecal total bacteria and *E. coli* populations showed significant quadratic relationships with dietary inulin supplementation level ($P<0.05$), with minimum populations observed at supplementation levels of 2.71 and 2.66 g/kg, respectively.

2.8 Rectal Microbial Populations in Broilers

As shown in , the populations of total bacteria and *E. coli* in groups III and V were significantly lower than in the control and groups II and IV ($P<0.05$). The rectal *Lactobacillus* population in group II was significantly higher than in all other groups ($P<0.05$). Group V showed the highest rectal *Bifidobacteria* population, which was significantly higher than in the control and groups III and IV ($P<0.05$). The rectal *C. perfringens* population in group V was significantly higher than in all other groups ($P<0.05$). Except for *E. coli*, all rectal bacterial populations showed significant linear relationships with inulin supplementation level ($P<0.05$). Additionally, rectal *Bifidobacteria* and *C. perfringens* populations showed significant quadratic relationships with dietary inulin supplementation level ($P<0.05$), with the minimum *Bifidobacteria* population observed at an inulin supplementation level of 2.96 g/kg.

3.1 Effects of Dietary Inulin Supplementation on Cecal and Rectal Microbial Community Structure

The diversity of intestinal microbiota can be comprehensively reflected by the position, number, and intensity of bands in PCR-DGGE profiles. Rehman et al. [**?**] reported that supplementation with 10 g/kg inulin in a corn-soybean meal

diet had no significant effect on the number of DGGE bands or microbial diversity indices in the jejunum and cecum of 42-day-old broilers. Yang et al. [**?**] found that dietary supplementation with 1.2 g/kg inulin did not significantly affect cecal microbial diversity, richness, or evenness. In vitro studies also confirmed that inulin supplementation had no significant effect on microbial diversity and richness in cecal and rectal chyme fermentation broth, although the number of DGGE bands decreased [**?**]. The present results showed that dietary inulin supplementation had no significant effect on the number of DGGE bands in cecal and rectal microbiota, but band numbers in all inulin-supplemented groups were lower than in the control group, and diversity indices were reduced. However, supplementation with certain levels of inulin significantly decreased cecal microbial evenness and rectal microbial Shannon-Wiener index, which slightly differs from previous reports. This discrepancy may be related to the limitations of PCR-DGGE technology, such as DNA extraction efficiency, DNA extraction and purification methods, and errors in PCR amplification. Additionally, DGGE technology can only detect and analyze dominant microbial populations. The cluster analysis and PCA in this study showed that cecal samples M1-2 and M1-3 from the control group and M5-1 and M5-3 from the 5.0 g/kg inulin group had a similarity coefficient of only 0.59. In rectal microbiota, Z5-2 showed the lowest similarity coefficient (0.27) with other samples. These results demonstrate that dietary inulin supplementation affected the composition of cecal and rectal microbiota in broilers. Sequence analysis of dominant bands in DGGE profiles revealed that different dominant bands appeared after inulin supplementation. Band 37 in cecal samples existed only in inulin-supplemented groups, and its sequence represented *Eubacterium coprostanoligenes* from the phylum Firmicutes, indicating that inulin supplementation promoted the growth of this bacterium. Bands 2, 33, 36, and 45 in rectal samples also existed only in inulin-supplemented groups, and their sequences represented cocci or clostridia from the phylum Firmicutes, indicating that inulin supplementation promoted the growth of these bacteria in the rectum. These results are generally consistent with Yang et al. [**?**], who reported that dietary supplementation with 1.2 g/kg inulin promoted the proliferation of uncultured Firmicutes Lachnospiraceae and uncultured Bacteroidetes.

3.2 Effects of Dietary Inulin Supplementation on Major Microbial Populations in Cecum and Rectum

Poultry intestinal microorganisms are generally divided into two categories: harmful bacteria (pathogens) and beneficial bacteria (pathogen inhibitors). Harmful bacteria mainly include *E. coli*, *Salmonella*, and *C. perfringens*, while *Lactobacillus* and *Bifidobacteria* are considered beneficial intestinal bacteria [**?**]. Increased beneficial bacteria reduce the fermentation of harmful bacteria in the intestine [**?**]. Results on the effects of dietary inulin supplementation on broiler intestinal microbial populations have been inconsistent. Lin [**?**] found that inulin could regulate broiler intestinal microbiota, with higher inulin levels resulting in fewer *E. coli* and more *Bifidobacteria* and *Lactobacillus*.

Nabizadeh [?] reported that dietary supplementation with 10 g/kg inulin significantly reduced cecal *E. coli* populations. Zhao et al. [?] found that dietary supplementation with 2.5 and 5.0 g/kg fructan significantly increased cecal *Lactobacillus* and *Bifidobacteria* populations, reduced cecal *E. coli* and *C. perfringens* populations, and decreased excreta ammonia emission. Kareem et al. [?] reported that dietary supplementation with postbiotics from *Lactobacillus* metabolism combined with 0.8% and 1.0% inulin significantly increased *Lactobacillus* populations in broiler excreta and reduced Enterobacteriaceae populations. However, other studies have shown no significant effects of inulin on intestinal microbial populations. Biggs et al. [?] reported that supplementation with 4 g/kg inulin in a corn-soybean meal diet had no significant effect on cecal *Lactobacillus*, *Bifidobacteria*, *C. perfringens*, and *E. coli* populations in 21-day-old broilers. Abdel-Raheem et al. [?] also demonstrated that probiotics and synbiotics had no significant effect on cecal *Lactobacillus* and *E. coli* populations in broilers. However, Abdelqader et al. [?] showed that inulin significantly reduced *E. coli* populations in the ileum and cecum of laying hens, although it did not significantly increase *Lactobacillus* and *Bifidobacteria* populations. The present results showed that dietary supplementation with 1.5, 2.5, and 5.0 g/kg inulin significantly reduced cecal *Lactobacillus* populations, while supplementation with 0.5 g/kg inulin significantly increased rectal *Lactobacillus* populations. Supplementation with 1.5 and 5.0 g/kg inulin significantly reduced cecal and rectal total bacteria and *E. coli* populations, while supplementation with 5.0 g/kg inulin significantly increased cecal and rectal *Bifidobacteria* populations. Dietary inulin supplementation had no significant effect on cecal *C. perfringens* populations, but supplementation with 5.0 g/kg inulin significantly increased rectal *C. perfringens* populations. These results are not entirely consistent with previous reports, which may be related to differences in broiler age, sex, sampling location, inulin source, structure, and dosage. Additionally, the probiotic effects of inulin are also greatly influenced by broiler rearing environment and individual factors [?]. In this study, the 5.0 g/kg inulin group promoted the proliferation of beneficial bacteria while also promoting the growth of harmful bacteria (*C. perfringens*) in the rectum, indicating that high-level inulin supplementation is not conducive to controlling harmful bacterial proliferation. The probiotic effect of inulin is mainly due to its special structure, which cannot be digested by digestive enzymes secreted by the poultry stomach and pancreas but enters the hindgut to be utilized by beneficial bacteria such as *Lactobacillus* and *Bifidobacteria*, producing short-chain fatty acids and lactic acid [?], thereby reducing the production of toxic metabolites (ammonia, indole, phenols, etc.) [?].

In summary, dietary inulin supplementation reduced indole and skatole concentrations in broiler excreta, cecum, and rectum [?] primarily by modulating microbial community structure, reducing total bacteria and *E. coli* populations, and increasing *Lactobacillus* and *Bifidobacteria* populations.

Conclusions

1. Appropriate dietary inulin supplementation significantly reduced cecal microbial evenness and rectal microbial Shannon-Wiener index in broilers, and promoted the proliferation of *Eubacterium coprostanoligenes*, *Intestinimonas sp.*, and *Enterococcus cecorum* from the phylum Firmicutes in the cecum and rectum.

2. Appropriate dietary inulin supplementation significantly reduced total bacterial and *E. coli* populations in the cecum and rectum, increased rectal *Lactobacillus* populations, and increased cecal and rectal *Bifidobacteria* and rectal *C. perfringens* populations.

3. Under the conditions of this experiment, the appropriate dietary inulin supplementation level for broilers was 2.5–5.0 g/kg.

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Note: Figure translations are in progress. See original paper for figures.